

Engineering Conferences International ECI Digital Archives

Integrated Continuous Biomanufacturing III

Proceedings

9-17-2017

Use of a biphasic perfusion process based on mild hypothermia for recombinant glucocerebrosidase (GBA) production

Filipa Gonçalves

Instituto Superior Técnico, Portugal

Juliana Coronel

Federal University of Rio de Janeiro (UFRJ)

Leda R. Castilho

Federal University of Rio de Janeiro (UFRJ)

Follow this and additional works at: http://dc.engconfintl.org/biomanufact_iii



Part of the [Engineering Commons](#)

Recommended Citation

Filipa Gonçalves, Juliana Coronel, and Leda R. Castilho, "Use of a biphasic perfusion process based on mild hypothermia for recombinant glucocerebrosidase (GBA) production" in "Integrated Continuous Biomanufacturing III", Suzanne Farid, University College London, United Kingdom Chetan Goudar, Amgen, USA Paula Alves, IBET, Portugal Veena Warikoo, Axcella Health, Inc., USA Eds, ECI Symposium Series, (2017). http://dc.engconfintl.org/biomanufact_iii/47

This Abstract and Presentation is brought to you for free and open access by the Proceedings at ECI Digital Archives. It has been accepted for inclusion in Integrated Continuous Biomanufacturing III by an authorized administrator of ECI Digital Archives. For more information, please contact franco@bepress.com.

Filipa M. Gonçalves^{1,2}, Juliana Coronel², Leda R. Castilho²

¹University of Lisbon, IST, Portugal; ²Federal University of Rio de Janeiro (UFRJ), COPPE, Cell Culture Engineering Lab, Brazil

Introduction

- Recombinant glucocerebrosidase (GBA): enzyme used for replacement therapy of Type I Gaucher disease, a lysosomal storage disorder
- Exposure of terminal mannose residues is important to increase GBA delivery to pathological macrophages: enzymatic deglycosylation of the commercially available CHO-derived Cerezyme® (Genzyme)
- Previous work at our laboratory (Gutierrez, 2010) developed GBA-producing clones derived from different CHO parental cell lines, including glycosylation mutants
- Focus of the present study: upstream process development based on evaluation of temperature reduction, supplementation of the culture medium with a productivity enhancer and perfusion
 - DOE to investigate mild hypothermic conditions and valeric acid supplementation for two cell lines (CHO-GBA-36K, CHO-GBA-65P)
 - Perfusion operation under the selected conditions, with the aim to maximize process productivity and product quality

Materials and Methods

Cell Culture

- CHO-GBA-36K and CHO-GBA-65P were cultivated in TC-LECC (Xell AG, Germany), a customized CD, ADCF medium
- A 2² DOE was performed in spinner flasks for each cell line (Table 1)

Table 1. DOE for evaluation of the effects of valeric acid supplementation and mild hypothermia.

Runs and replicates	Valeric Acid	Temperature
[1] (n=2)	-1 (0 mM)	-1 (31 °C)
[2] (n=2)	-1 (0 mM)	+1 (37 °C)
[3] (n=2)	+1 (2 mM)	-1 (31 °C)
[4] (n=2)	+1 (2 mM)	+1 (37 °C)
[5] (n=3)	0 (1 mM)	0 (34 °C)

- Perfusion process
 - stirred tank bioreactor (RALF, BioEngineering AG)
 - pH 7.1, 150 rpm, 40% air saturation, 37°C with shift to 31°C (d7)
 - CS-10 inclined settler (Biotechnology Solutions)
 - perfusion start when target viable cell concentration reached
 - dilution rate in increasing steps: 0.5, 0.75, 1 and 2 vvd
- Analytical
 - Viable cell density (VCD) and cell viability were determined by trypan blue exclusion method using a Neubauer chamber in an optical microscope (Eclipse TS100, Nikon) or using an automatic counter equipment (Vi-Cell XR, Beckman Coulter)
 - GBA enzyme activity in the samples was determined by measuring the release of the fluorescent compound 4-methylumbelliferone (4-MU) upon hydrolysis of the synthetic substrate 4-methylumbelliferyl-β-D-glucopyranoside (4-MUD, Sigma, M3633). The fluorescent product was quantified with a fluorescence microplate reader (Victor III, Perkin Elmer) using excitation and emission filters of 355 nm and 460 nm, respectively
 - Glucose and lactate were measured using a YSI2700 analyser (Yellow Springs Instruments)

Conclusions

- For both cell clones, a subphysiological temperature of 31°C led to an increase in the specific productivity (q_p), but the effect of valeric acid supplementation was clone dependent.
- The best overall performance regarding both cell growth and productivity was obtained for the CHO-GBA-65P cell clone at 31°C without valeric acid.
- The maximum product titer achieved in perfusion was 9.5-fold higher compared to batch at 31°C and 22-fold higher than the control batch (at 37°C).
- Perfusion typically results in higher volumetric productivities but lower titers. However, for this enzyme, our results show that a biphasic perfusion strategy including a temperature downshift can significantly enhance also GBA titer in the harvest, which is an advantage for subsequent downstream processing.

Financial support from the Brazilian research funding agencies CNPq, Capes and Faperj is gratefully acknowledged.

Results

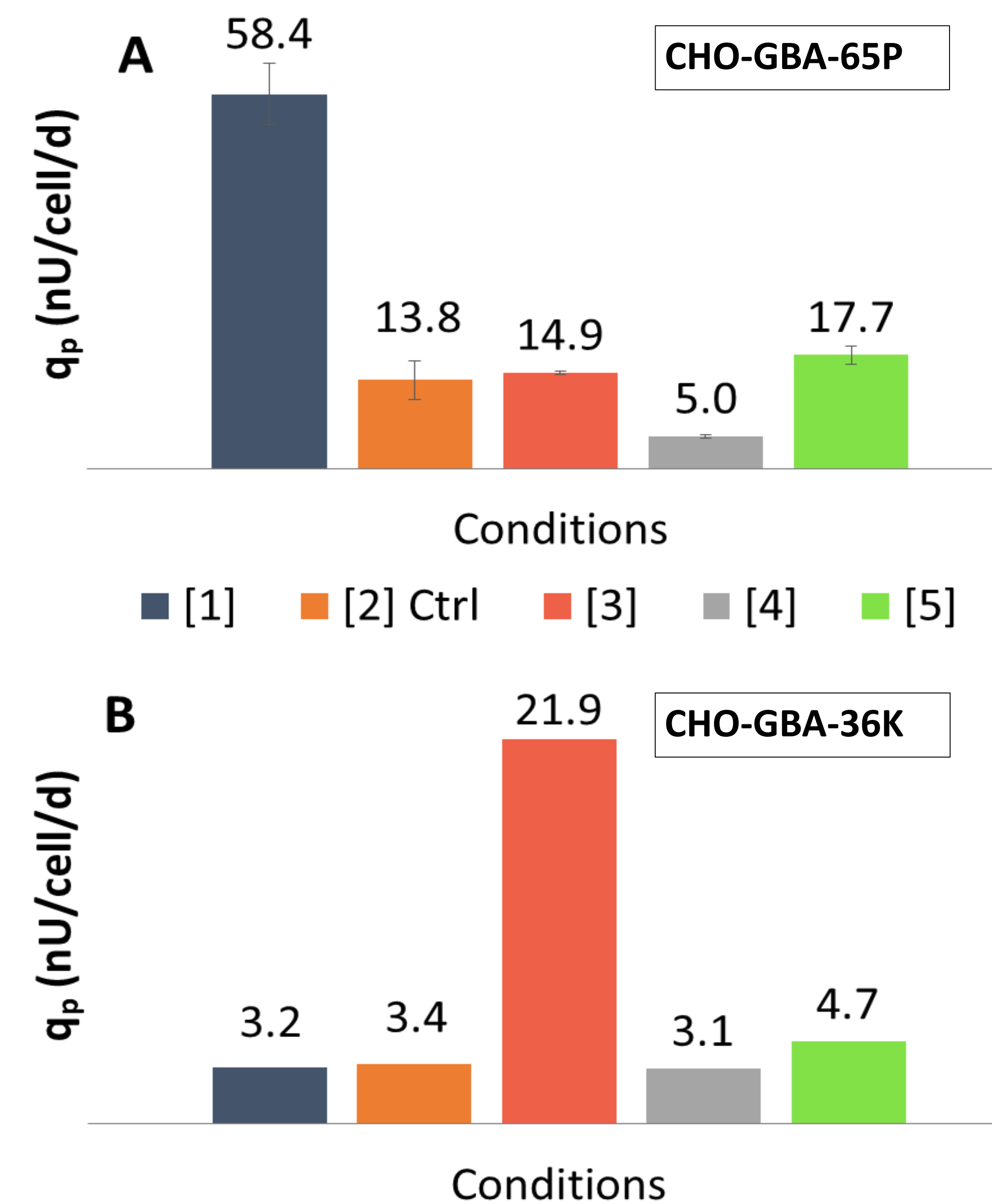


Fig 1. Specific productivity (q_p) for the different DOE conditions using CHO-GBA-65P (A) and CHO-GBA-36K (B) cells. Under condition [1] (31°C, no valeric acid), CHO-GBA-65P achieved a maximum q_p of 58.4 nU/cell/d, which was 4.2 fold higher than q_p at the control condition [2] and 2.7 fold higher than the maximum q_p obtained for the CHO-GBA-36K clone, which was achieved at 31°C with 2 mM valeric acid supplementation (condition [3]).

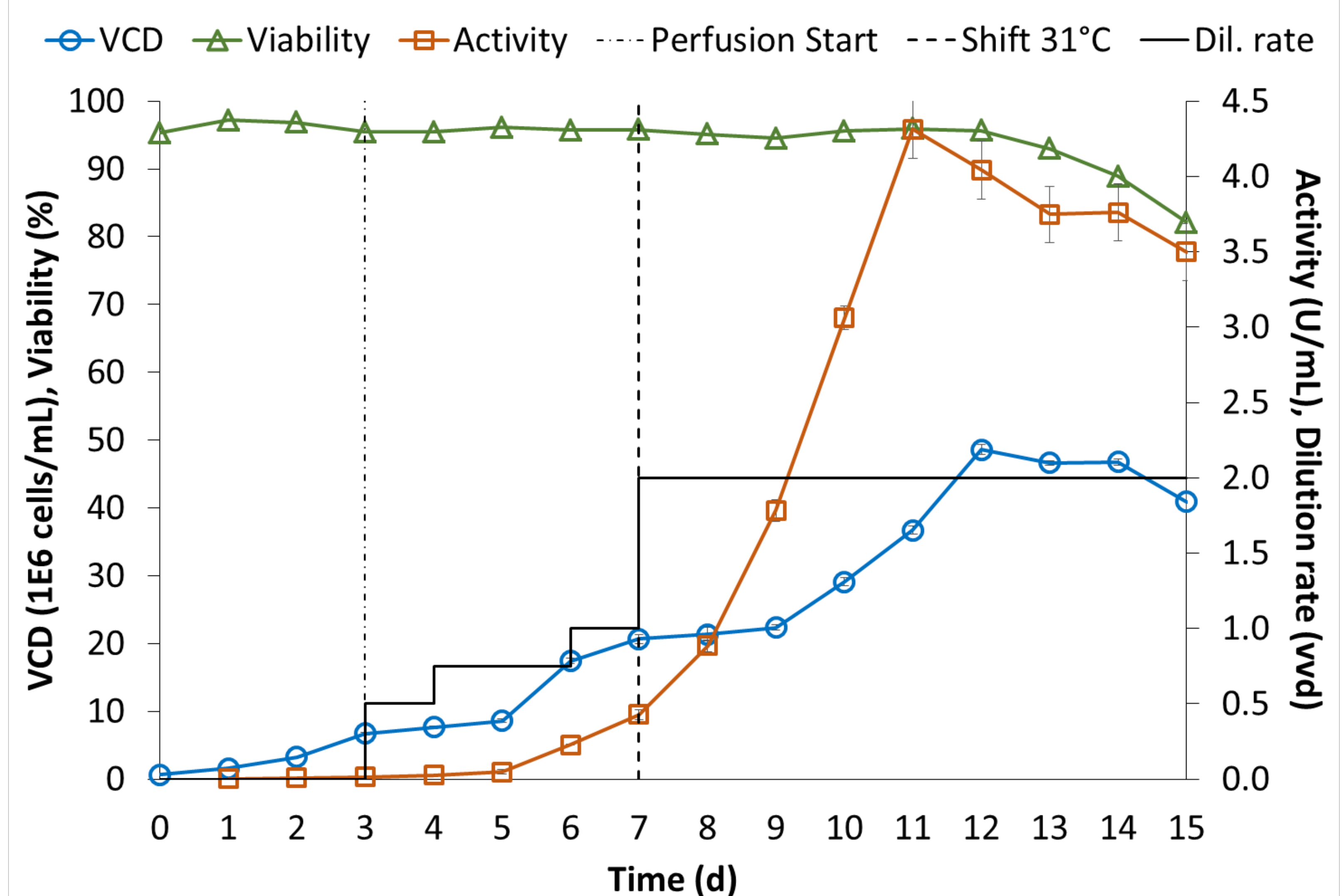


Fig 2. Perfusion process using CHO-GBA-65P clone. Perfusion was started on day 3, and a temperature downshift to 31°C was applied on day 7. The application of a perfusion rate of 2 vvd enabled cell densities up to 50E6 cells/mL to be achieved.